

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
17 January 2002 (17.01.2002)

PCT

(10) International Publication Number
WO 02/04475 A1

- (51) International Patent Classification⁷: **C07H 19/20**, A61K 31/7076, A61P 9/00 (74) Agents: **BERNSTEIN, Mitchell** et al.; Darby & Darby P.C., 805 Third Avenue, New York, NY 10022-7513 (US).
- (21) International Application Number: PCT/US01/21523 (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (22) International Filing Date: 6 July 2001 (06.07.2001)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data: 60/216,869 7 July 2000 (07.07.2000) US (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- (71) Applicant (*for all designated States except US*): **THE RESEARCH FOUNDATION OF STATE UNIVERSITY OF NEW YORK** [US/US]; Stony Brook, NY 11794-3366 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): **JOHNSON, Roger, A.** [US/US]; 7 Bayview Avenue, East Setauket, NY 11733-3903 (US). **PANDE, Praveen** [US/US]; 220 Pointe Circle, Coram, NY 11727 (US). **LAUX, Wolfgang** [DE/DE]; Sachsenhaeuser Landwehrweg 87, D-60599 Frankfurt am Main (DE). **GOSSELIN, Gilles** [FR/FR]; Parc des Arceaux, Batiment F1, 83 Rue Calvin, F-34080 Montpellier (FR).
- Published:**
- with international search report
 - before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*



WO 02/04475 A1

(54) Title: 9-SUBSTITUTED ADENINE DERIVATIVES AS PRODRUG REGULATORS OF CELL AND TISSUE FUNCTION

(57) Abstract: The present invention relates to 9-substituted adenine derivatives that inhibit adenylyl cyclase in cells but have little or no effect on isolated adenylyl cyclase. The derivatives are useful as prodrugs for inhibiting adenylyl cyclase and lowering 3':5'-cAMP in cells, thereby inhibiting adenylyl cyclase dependent effects within cells.

9-SUBSTITUTED ADENINE DERIVATIVES AS PRODRUG
REGULATORS OF CELL AND TISSUE FUNCTION

FIELD OF THE INVENTION

5 The present invention is directed to novel derivatives of adenine that inhibit adenylyl cyclase in cells but have little or no effect on isolated adenylyl cyclase. The present invention is also directed to methods for the use of these compounds to inhibit adenylyl cyclase dependent effects within cells.

BACKGROUND OF THE INVENTION

10 Adenosine-3':5'-cyclic monophosphate (3':5'-cAMP) is the second messenger mediating the transmembrane actions of numerous agents throughout the animal kingdom. It acts typically through the activation of 3':5'-cAMP-dependent protein kinases (PKA), which catalyze the phosphorylation of specific proteins [1,2], or through effects on 3':5'-cAMP-gated ion channels [3,4]. Consequently,
15 relationships between 3':5'-cAMP and cell function are typically viewed as effects mediated by these two mechanisms. The 3':5'-cAMP signaling pathway can be regulated pharmacologically by many drugs that are of particular value in the treatment of various diseases and therefore there is continuing interest in identifying
20 new agents acting on this pathway.

20 Cellular levels of 3':5'-cAMP are regulated by adenylyl cyclase, a family of membrane-bound enzymes that catalyze the formation of 3':5'-cAMP from

5'-ATP [5,6], and cyclic nucleotide phosphodiesterases, a family of enzymes that catalyze conversion of 3':5'-cAMP to 5'-AMP [7,8]. Agents that affect cellular 3':5'-cAMP levels, whether by affecting the activity of cyclic nucleotide phosphodiesterases or of adenylyl cyclases, also will typically affect changes in cell function.

Mammalian forms of adenylyl cyclase are a family of membrane-bound enzymes that catalyze the formation of 3':5'-cAMP from 5'-ATP. The family includes at least 10 isozymes and their expression is tissue dependent and developmentally variable. Activities of adenylyl cyclases are regulated by numerous neurotransmitters, autacoids, and hormones via cell surface receptors that act through heterotrimeric guanine nucleotide-dependent regulatory proteins (G-proteins; comprising α , β , and γ subunits). G-proteins may be either stimulatory (G_s) [9] or inhibitory (G_i) for adenylyl cyclase activity, and may be either specific or promiscuous in effecting activity of different isozymes [9-11]. $G_s\alpha$, for example, activates all isozymes, possibly save the isozyme from sperm. Adenylyl cyclase activity may also be altered by other agents of physiological and biochemical interest, including bacterial toxins that act on G_s and G_i , agents or enzymes that act on hormone receptors, and agents that act directly on adenylyl cyclase, e.g. Ca^{2+} /calmodulin [12], forskolin [13,14], certain oxidants [15,16], protein kinases [17], and various adenine derivatives.

Numerous drugs have been developed as therapeutic agents that inhibit cyclic nucleotide phosphodiesterases [8]. There are also numerous therapeutically useful drugs that activate or inhibit adenylyl cyclases indirectly. One class of therapeutic agents having an indirect effect on adenylyl cyclases includes agents that are receptor blockers, such as agents that, for example, block receptors for catecholamines (dopamine, norepinephrine, and epinephrine), angiotensin II, adenosine and other purines. β -blockers that are commonly used to treat hypertension, for example, act to inhibit adenylyl cyclase indirectly by blocking the stimulatory effects of the sympathetic nervous system to activate adenylyl cyclase in the heart [5], in which the type V isozyme is expressed predominantly [6]. By comparison to drugs that inhibit cyclic nucleotide phosphodiesterases or that affect

adenylyl cyclases indirectly, drugs that act directly on adenylyl cyclases are uncommon. The main class of such agents comprises analogs of forskolin [13,14].

Drugs that inhibit adenylyl cyclase directly would be particularly highly prized. The expectation is that such agents that act downstream of cell-surface
5 receptors to inhibit adenylyl cyclase directly should have a cardiac-sparing effect and be useful in reducing cardiomyopathies and heart failure. We have developed a means to block the formation of 3':5'-cAMP in intact tissues by a unique class of specific and selective nucleotide prodrugs. These nucleotide prodrugs will find extensive use as pharmacologically and biochemically useful inhibitors of adenylyl cyclase. The
10 prodrugs exhibit potencies in the nanomolar range and have shown their usefulness to regulate function in isolated cells and in intact tissues. Thus, the prodrugs of the invention are the most potent, directly acting inhibitors of adenylyl cyclases in tissues, and represent a new and unique class of drug with therapeutic potential.

Adenylyl cyclases are inhibited by a number of polyphosphorylated
15 derivatives of adenine and adenosine (Table 1). Biochemical assays have been used to characterize inhibition of adenylyl cyclases by adenine derivatives with respect to ligand structures and inhibition kinetics [18-35]. The enzyme is inhibited competitively by substrate analogs that interact with a pre-transition configuration of the enzyme [31]. The most potent of these is β -L-2',3'-dd-5'-ATP (Table 1 and Fig.
20 1). In contrast, inhibition by adenine nucleoside 3'-phosphates bind to a post-transition configuration and inhibition is uncompetitive or non-competitive, depending on the mode of activation [25, 26, 33]. Potent inhibitors that bind selectively to pre- and post-transition states are extremely uncommon and because of this these compounds may form the basis of new inhibitors with unique biochemical
25 and pharmacological properties.

3'-Phosphate	NUCLEOSIDE (IC ₅₀ , μ M)		
	Ado	2'dAdo	2',5'ddAdo
none	82	15	2.7
3'~P	8.9	1.2	0.46
3'~PP	3.9	0.14	0.10
3'~PPP	2.0	0.09	0.040
3'~PPPP	--	0.0105	0.0074
3'~PS	--	3.1	0.60
<hr/>			
Substrate analogs:			IC ₅₀ (μ M)
β -L-5'-AMP			200
β -L-2',3'-dd-5'-AMP			62
β -L-5'-ATP			3.2
β -L-2',3'-dd-5'-ATP			0.024
<hr/>			
PMEA-mimics:			IC ₅₀ (μ M)
PMEA			65
PMEApp			0.17
PMEAp(NH)p			0.18
<hr/>			
From Johnson and coll. [27-32]			

Table 1: IC₅₀s for rat brain adenylyl cyclase

The acyclic phosphonate derivatives of adenine, typified by PMEApp [32], are somewhat less potent than β -L-2',3'-dd-5'-ATP or 2',5'-dd-3'-ATP (Table 1), but the kinetic behavior of these agents conformed to mixed inhibition, i.e., kinetic behavior sharing aspects with that seen with the competitive and noncompetitive inhibitors. These distinctions form the basis for the rational design of compounds that can effect inhibition of adenylyl cyclases in intact tissues.

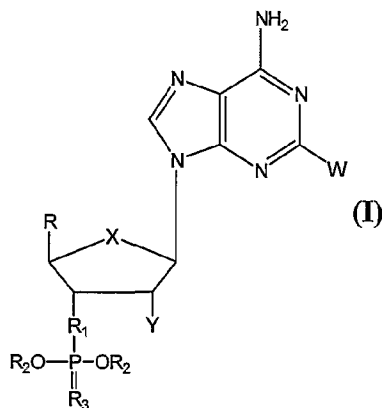
Crystal structures of the adenylyl cyclase catalytic cleft were solved in both pre- and post-transition states by use of β -L-2',3'-dd-5'-ATP and 2',5'-dd-3'-ATP (Figure 2) [36,37]. Note that there are only small differences in the topology of the catalytic cleft into which the respective ligands bind. It is presumed that PMEApp and its mimics bind in this same locus.

There are a number of cell-permeable derivatives of adenine that have been used to lower cellular 3':5'-cAMP levels and to alter function in isolated cells and intact tissues. These include 2',5'-dideoxyadenosine (2',5'-dd-Ado), 9-(tetrahydrofuryl)-adenine (9-THF-Ade;), 9-(arabinofuranosyl)-adenine (9-Ara-Ade), or 9-(cyclopentyl)-adenine (9-CP-Ade). These compounds are typically effective at

concentrations from 10 to 100 μ M. They have been used with epididymal fat cells [38], isolated hepatocytes [39], pre-adipocytes [40], thyroid follicles [41], dorsal root ganglion neurons [42], bone organ cultures [43], cortical collecting tubules [44], phagocytes [45], and platelets [20], to name but a few. End-points included cell differentiation [40], water conductance [44], action potential after-hyperpolarization [42], bone resorption [43], glycerol production [38], altered enzyme activity [39], DNA synthesis and cell growth [41], and FC γ -receptor-mediated phagocytosis [45]. Effects of these agents on cell function were uniformly consistent with inhibition of adenylyl cyclase by these ligands. The expectation is that the compounds of the invention will also affect cell function in these systems, but be substantially more potent in doing so.

SUMMARY OF THE INVENTION

The invention relates to 9-substituted adenine derivatives prodrugs that inhibit adenylyl cyclase in cells but have little or no effect on isolated adenylyl cyclase. The compounds preferably have a structure of formula (I) below:



wherein W is selected from the group consisting of H, halogen, azido and amino group;
 X is selected from the group consisting of O, S, N(H) and C;
 Y is selected from the group consisting of H, hydroxy, C₁₋₄ alkyl, C₁₋₄ alkoxy and halogen;
 R is selected from the group consisting of H, hydroxymethyl, C₁₋₄ alkyl, C₁₋₄ alkoxy and halogen;

R_1 is selected from the group consisting of O, NH and CH_2 ;

R_2 represents a radical selected from the group consisting of $-(CH_2)_n-S-C(O)-R_4$ and $-(CH_2)_n-S-S-R_4$, where $n=1-4$ and R_4 is a C_{1-4} alkyl or aryl group and R_4 is optionally substituted with a halogen, amino, N-alkylamino, N, N di-alkylamino or C_{1-4} alkoxy group, and wherein each of the R_2 radicals may be the same or different;

R_3 is O or S; and

when X is C the cyclopentyl ring so formed may be saturated or unsaturated.

The following are independent preferences.

The preferred group that W represents is H.

A preferred alkoxy group that Y or R represents is methoxy. A preferred alkyl group that Y or R represents is methyl.

Also preferred is where R_4 is selected from the group consisting of unsubstituted C_{1-4} alkyl and unsubstituted aryl groups. Still more preferred is where R_4 is selected from the group consisting of methyl, tert-butyl and phenyl groups.

The preferred value for n is 2.

Also preferred is where, simultaneously, Y is selected from the group consisting of H, hydroxy, methyl, methoxy and halogen; and R is selected from the group consisting of H, methyl, hydroxymethyl, methoxy and halogen.

Still more preferred is where, simultaneously, Y is selected from the group consisting of H, hydroxy, methyl, methoxy and halogen; R is selected from the group consisting of H, methyl, hydroxymethyl, methoxy and halogen; R_4 is selected from the group consisting of methyl, tert-butyl and phenyl; and $n=2$.

Still more preferred is where, simultaneously, W represents a hydrogen atom; Y is selected from the group consisting of H, hydroxy, methyl, methoxy and halogen; R is selected from the group consisting of H, methyl, hydroxymethyl, methoxy and halogen; R_4 is selected from the group consisting of methyl, tert-butyl and phenyl; and $n=2$.

In certain embodiments, a compound of the invention is selected from the group consisting of:

2',5'-dideoxy- β -(L)-adenosine 3'-monophosphate-bis(Me-SATE);

2',5'-dideoxy- β -(D)-adenosine 3'-monophosphate-bis(Me-SATE);

- 2'-deoxy- β -(L)-adenosine 3'-monophosphate-bis(Me-SATE);
2'-deoxy- β -(D)-adenosine 3'-monophosphate-bis(Me-SATE);
2',5'-dideoxy- β -(L)-adenosine 3'-monophosphate-bis(t-Bu-SATE);
2',5'-dideoxy- β -(D)-adenosine 3'-monophosphate-bis(t-Bu-SATE);
5 2'-deoxy- β -(L)-adenosine 3'-monophosphate-bis(t-Bu-SATE);
2'-deoxy- β -(D)-adenosine 3'-monophosphate-bis(t-Bu-SATE);
2',5'-dideoxy- β -(L)-adenosine 3'-monophosphate-bis(Ph-SATE);
2',5'-dideoxy- β -(D)-adenosine 3'-monophosphate-bis(Ph-SATE);
2'-deoxy- β -(L)-adenosine 3'-monophosphate-bis(Ph-SATE);
10 2'-deoxy- β -(D)-adenosine 3'-monophosphate-bis(Ph-SATE);
2',5'-dideoxy- β -(L)-adenosine 3'-methylene phosphate-bis(Me-SATE);
2',5'-dideoxy- β -(D)-adenosine 3'-methylene phosphate-bis(Me-SATE);
2'-deoxy- β -(L)-adenosine 3'-methylene phosphate-bis(Me-SATE);
2'-deoxy- β -(D)-adenosine 3'-methylene phosphate-bis(Me-SATE);
15 2',5'-dideoxy- β -(L)-adenosine 3'-methylene phosphate-bis(t-Bu-SATE);
2',5'-dideoxy- β -(D)-adenosine 3'-methylene phosphate-bis(t-Bu-SATE);
2'-deoxy- β -(L)-adenosine 3'-methylene phosphate-bis(t-Bu-SATE);
2'-deoxy- β -(D)-adenosine 3'-methylene phosphate-bis(t-Bu-SATE);
2',5'-dideoxy- β -(L)-adenosine 3'-methylene phosphate-bis(Ph-SATE);
20 2',5'-dideoxy- β -(D)-adenosine 3'-methylene phosphate-bis(Ph-SATE);
2'-deoxy- β -(L)-adenosine 3'-methylene phosphate-bis(Ph-SATE);
2'-deoxy- β -(D)-adenosine 3'-methylene phosphate-bis(Ph-SATE);
2',5'-dideoxy- β -(L)-adenosine 3'-iminophosphate-bis(Me-SATE);
2',5'-dideoxy- β -(D)-adenosine 3'-iminophosphate-bis(Me-SATE);
25 2'-deoxy- β -(L)-adenosine 3'-iminophosphate-bis(Me-SATE);
2'-deoxy- β -(D)-adenosine 3'-iminophosphate-bis(Me-SATE);
2',5'-dideoxy- β -(L)-adenosine 3'-iminophosphate-bis(t-Bu-SATE);
2',5'-dideoxy- β -(D)-adenosine 3'-iminophosphate-bis(t-Bu-SATE);
2'-deoxy- β -(L)-adenosine 3'-iminophosphate-bis(t-Bu-SATE);
30 2'-deoxy- β -(D)-adenosine 3'-iminophosphate-bis(t-Bu-SATE);
2',5'-dideoxy- β -(L)-adenosine 3'-iminophosphate-bis(Ph-SATE);

2',5'-dideoxy- β -(D)-adenosine 3'-iminophosphate-bis(Ph-SATE);
 2'-deoxy- β -(L)-adenosine 3'-iminophosphate-bis(Ph-SATE); and
 2'-deoxy- β -(D)-adenosine 3'-iminophosphate-bis(Ph-SATE).

In other embodiments, a compound of the invention is selected from

- 5 the group consisting of :
- 9-(tetrahydrofuryl)-(1'R,3'S)-adenine-3'-phosphate-bis(Me-SATE);
 - 9-(tetrahydrofuryl)-(1'R,3'R)-adenine-3'-phosphate-bis(Me-SATE);
 - 9-(tetrahydrofuryl)-(1'R,3'S)-adenine-3'-phosphate-bis(t-Bu-SATE);
 - 9-(tetrahydrofuryl)-(1'R,3'R)-adenine-3'-phosphate-bis(t-Bu-SATE);
 - 10 9-(tetrahydrofuryl)-(1'R,3'S)-adenine-3'-phosphate-bis(Ph-SATE);
 - 9-(tetrahydrofuryl)-(1'R,3'R)-adenine-3'-phosphate-bis(Ph-SATE);
 - 9-(tetrahydrofuryl)-(1'R,3'S)-adenine-3'-(CH₂)-phosphate-bis(Me-SATE);
 - 9-(tetrahydrofuryl)-(1'R,3'R)-adenine-3'-(CH₂)-phosphate-bis(Me-SATE);
 - 9-(tetrahydrofuryl)-(1'R,3'S)-adenine-3'-(CH₂)-phosphate-bis(t-Bu-SATE);
 - 15 9-(tetrahydrofuryl)-(1'R,3'R)-adenine-3'-(CH₂)-phosphate-bis(t-Bu-SATE);
 - 9-(tetrahydrofuryl)-(1'R,3'S)-adenine-3'-(CH₂)-phosphate-bis(t-Ph-SATE);
 - 9-(tetrahydrofuryl)-(1'R,3'R)-adenine-3'-(CH₂)-phosphate-bis(t-Ph-SATE);
 - 9-(tetrahydrofuryl)-(1'R,3'S)-adenine-3'-(NH)-phosphate-bis(Me-SATE);
 - 9-(tetrahydrofuryl)-(1'R,3'R)-adenine-3'-(NH)-phosphate-bis(Me-SATE);
 - 20 9-(tetrahydrofuryl)-(1'R,3'S)-adenine-3'-(NH)-phosphate-bis(t-Bu-SATE);
 - 9-(tetrahydrofuryl)-(1'R,3'R)-adenine-3'-(NH)-phosphate-bis(t-Bu-SATE);
 - 9-(tetrahydrofuryl)-(1'R,3'S)-adenine-3'-(NH)-phosphate-bis(Ph-SATE); and
 - 9-(tetrahydrofuryl)-(1'R,3'R)-adenine-3'-(NH)-phosphate-bis(Ph-SATE).

In still other embodiments of the invention, a compound is selected

- 25 from the group consisting of:
- 9-(cyclopentyl)-(1'R,3'S)-adenine-3'-phosphate-bis(Me-SATE);
 - 9-(cyclopentyl)-(1'R,3'R)-adenine-3'-phosphate-bis(Me-SATE);
 - 9-(cyclopentyl)-(1'R,3'S)-adenine-3'-phosphate-bis(t-Bu-SATE);
 - 9-(cyclopentyl)-(1'R,3'R)-adenine-3'-phosphate-bis(t-Bu-SATE);
 - 30 9-(cyclopentyl)-(1'R,3'S)-adenine-3'-phosphate-bis(Ph-SATE);
 - 9-(cyclopentyl)-(1'R,3'R)-adenine-3'-phosphate-bis(Ph-SATE);

- 9-(cyclopentyl)-(1'R,3'S)-adenine-3'-(CH₂)-phosphate-bis(Me-SATE);
 9-(cyclopentyl)-(1'R,3'R)-adenine-3'-(CH₂)-phosphate-bis(Me-SATE);
 9-(cyclopentyl)-(1'R,3'S)-adenine-3'-(CH₂)-phosphate-bis(t-Bu-SATE);
 9-(cyclopentyl)-(1'R,3'R)-adenine-3'-(CH₂)-phosphate-bis(t-Bu-SATE);
 5 9-(cyclopentyl)-(1'R,3'S)-adenine-3'-(CH₂)-phosphate-bis(t-Ph-SATE);
 9-(cyclopentyl)-(1'R,3'R)-adenine-3'-(CH₂)-phosphate-bis(t-Ph-SATE);
 9-(cyclopentyl)-(1'R,3'S)-adenine-3'-(NH)-phosphate-bis(Me-SATE);
 9-(cyclopentyl)-(1'R,3'R)-adenine-3'-(NH)-phosphate-bis(Me-SATE);
 9-(cyclopentyl)-(1'R,3'S)-adenine-3'-(NH)-phosphate-bis(t-Bu-SATE);
 10 9-(cyclopentyl)-(1'R,3'R)-adenine-3'-(NH)-phosphate-bis(t-Bu-SATE);
 9-(cyclopentyl)-(1'R,3'S)-adenine-3'-(NH)-phosphate-bis(Ph-SATE); and
 9-(cyclopentyl)-(1'R,3'R)-adenine-3'-(NH)-phosphate-bis(Ph-SATE).

In another aspect, the invention provides methods of reducing or inhibiting adenylyl cyclase activity in intact cells and tissues comprising the steps of
 15 contacting cells or tissues with a compound of the invention for a period of time and under such conditions such that the adenylyl cyclase activity is reduced or inhibited.

In yet another aspect, the invention provides compounds, compositions and methods for regulating cardiac function or performance.

The invention also provides methods of altering the growth,
 20 development or differentiation of cells and tissues by administering a compound of the invention.

Naturally, as used herein, administration of a compound of the invention is meant to encompass the instances where a compound of the invention is administered alone, in combination with one or more other compounds of the
 25 invention or in conjunction with other compounds.

BRIEF DESCRIPTIONS OF THE DRAWINGS

Fig. 1 illustrates the catalytic intermediates and points of inhibition by adenine compounds for adenylyl cyclase.

30 Fig. 2A-C illustrates the binding of adenine derivatives in the active site of adenylyl cyclase.

Fig. 3 illustrates a mechanism of inhibition of adenylyl cyclase by 2',5'-dd-β-L-3'-AMP-bis(t-Bu-SATE) prodrug.

Fig. 4 illustrates the structures of 9-substituted adenine prodrugs.

Fig. 5 illustrates synthetic schemes for cyclosaligenyl-derivatives and
5 β-L-2',5'-dd-3'-AMP-bis(tBu-SATE).

Fig. 6 illustrates 9-substituted adenine prodrug inhibition of forskolin-stimulated 3':5'-cAMP formation in isolated cells.

Fig. 7 illustrates the effect of phenylephrine on contractility in rat atria.

Fig. 8 illustrates the effects of 10 μM 2',5'-dd-β-D-3'-AMP-bis(t-Bu-
10 SATE) on cardiac responses to 10 μM phenylephrine, added once as indicated, and 10 μM forskolin, added twice as indicated.

DETAILED DESCRIPTION OF THE INVENTION

All U.S. patents and other publications cited herein are hereby
15 incorporated by reference in their entirety. In the case of inconsistencies in definitions, the present description will control.

It is further understood that all compounds described, listed and represented herein are meant to include all hydrates, solvates, polymorphs and pharmaceutically acceptable salts thereof.

20 Some compounds described herein contain one or more asymmetric centers and may thus give rise to diastereomers and optical isomers. The present invention is meant to comprehend such possible diastereomers as well as their racemic and resolved, enantiomerically pure forms and pharmaceutically acceptable salts thereof.

25 Some of the compounds described herein contain olefinic double bonds and, unless otherwise specified, are meant to include both E and Z geometric isomers.

Because tissues and cells are typically impermeable to charged molecules, phosphorylated adenine derivatives are generally taken up poorly by cells and so typically they exhibit little effect on cells. To circumvent this problem, we
30 applied techniques currently being used in the development of nucleoside-based

antiviral therapeutics to create cell permeable precursors of adenylyl cyclase inhibitors. The idea is that these compounds, which are inactive on the isolated enzyme, will enter cells and be metabolically converted into more potent compounds that then effect changes in cell function. Nucleotide prodrugs have protected
5 phosphate groups to afford the molecule cell permeability. Once a protected nucleoside monophosphate is inside the cell the protecting group is cleaved and the nucleotide is then converted to a more potent form by intracellular enzymes (Figure 3 and Gosselin et al., U.S. Patent No. 5,849,905).

In preferred embodiments, protection of phosphorylated compounds
10 can be achieved through thio-ethyl linkages. One protection group is, for example, the S-acyl-thioethyl group, where the acyl group may be any of several moieties, e.g. tert-butylacyl, methylacyl or phenylacyl, etc. This agent undergoes cleavage by carboxyesterases at the S-acyl bond, thereby allowing the intramolecular attack of the sulfur on the phosphate group to yield the leaving of the thio-ethyl group. The
15 phosphate group is then free to participate in other reactions within the cell, e.g. phosphorylation.

A parallel set of reactions occurs if an alkylthioethyl group is used as protective ligand. In this instance cleavage occurs between the two sulfur atoms by the action of cellular reductases. The resultant free thiol may then undergo
20 intramolecular attack of phosphate group with subsequent leaving of the thioethyl group and freeing of the phosphate group [50, 55].

U.S. Patent Nos. 5,770,725 and 5,849,905 to Gosselin et al. disclose biologically active phosphotriester-type nucleosides and methods for preparing the same. U.S. Patent No. 5,795,756 to Johnson et al. discloses a method and compounds
25 for the inhibition of adenylyl cyclase.

Though there are many prodrug derivatives of adenine and adenosine with potential as intracellular inhibitors of adenylyl cyclases, we found that only one group of compounds exhibited the appropriate behavior of acting as very weak inhibitors of adenylyl cyclase when tested on isolated enzyme but having potent
30 effects when tested on intact cells. The prodrugs of the invention are among the most potent agents for inhibiting adenylyl cyclase dependent events in cells.

The prodrugs of the invention comprise the base adenine.

Substitutions on the adenine base will typically compromise the ability of a prodrug or a de-protected prodrug-derivative to act as an inhibitor of adenylyl cyclase. However, adenine derivatives substituted at the 2-position of the adenine base with an amino,
 5 cyano or halogen atom may retain adenylyl cyclase inhibitory activity.

Families of ligands were made based on derivatives of:

i) 9-(phosphonomethoxyethyl)-adenine (PMEA); ii) β -L-adenosine; and iii) 2',5'-dideoxy adenosine (2',5'-dd-Ado).

Prodrug structures. We have adapted the following protecting substituents
 10 for derivatization of adenine:

- i) *tert*-butyl-(S-acyl-2-thioethyl); (t-Bu-SATE-)
- ii) methyl-(S-acyl-2-thioethyl); (Me-SATE-)
- iii) phenyl-(S-acyl-2-thioethyl); (Ph-SATE-)
- iv) cyclosalicyl; (H-Sal-)
- 15 v) 3-methyl-cyclosalicyl; (Me-Sal-)

These will yield the following compounds (Figure 4):

- 1) 2',5'-dd-3'-AMP-bis(Me-SATE)
- 2) 2',5'-dd-3'-AMP-bis(t-Bu-SATE)
- 3) 2',5'-dd-3'-AMP-bis(Ph-SATE)
- 20 4) β -L-2',5'-dd-3'-AMP-bis(Me-SATE)
- 5) β -L-2',5'-dd-3'-AMP-bis(t-Bu-SATE)
- 6) β -L-2',5'-dd-3'-AMP-bis(Ph-SATE)
- 7) β -L-2',3'-dd-5'-AMP-bis(Me-SATE)
- 8) β -L-2',3'-dd-5'-AMP-bis(tBu-SATE)
- 25 9) β -L-2',3'-dd-5'-AMP-bis(Ph-SATE)
- 10) 2',5'-dd-3'-AMP-(H-Sal)
- 11) 2',5'-dd-3'-AMP-(Me-Sal)
- 12) bis(Me-SATE)-9-(2-phosphonomethoxyethyl)-adenine; (Me-SATE-PMEA)
- [46]
- 30 13) bis(t-Bu-SATE)-9-(2-phosphonomethoxyethyl)-adenine;

(t-Bu-SATE-PMEA)[46]

14) bis(Ph-SATE)-9-(2-phosphonomethoxyethyl)-adenine; (Ph-SATE-PMEA) [46]

15) bis(Me-SATE)-9-(2-phosphonomethoxypropyl)-adenine; (Me-SATE-PMPA)

16) bis(t-Bu-SATE)-9-(2-phosphonomethoxypropyl)-adenine;

5 (t-Bu-SATE-PMPA)

17) bis(Ph-SATE)-9-(2-phosphonomethoxypropyl)-adenine; (Ph-SATE-PMPA)

Of these 1), 2), 3), 5), 8), 10), 11), 12), 13), 14), and 16) have been synthesized.

10 The examples shown below illustrate the invention as described in this text, with no intention to limit it.

EXAMPLE 1 Prodrug Syntheses

a. 2',5'-dideoxyadenosine-3'-O-bis(S-acyl-2-thioethyl)-phosphates

15 (compounds 1-3 in Fig. 4). To a stirred solution of 2',5'-dideoxyadenosine (100 mg, 0.42 mmol) in a mixture of tetrahydrofuran and dimethylformamide (1.5 ml, 1:0.5, v/v) was added 1*H*-tetrazole (88 mg, 1.26 mmol, 3.0 eq) followed by the dropwise addition at room temperature of a solution of the bis(S2-hydroxyethylthioacyl) N, N-diisopropylphosphoramidite (0.51 mmol, 1.25 eq of -acetyl-: 188 mg (for compound 1); -pivaloyl-: 230 mg (for compound 2); or -benzoyl-: 252 mg (for compound 3)) in 20 0.5 ml tetrahydrofuran. The reaction mixture was stirred for 45 min and then cooled to -40°C and *tert*-butylhydroperoxide (0.44 ml, 1.26 mmol, 3.0 eq, 90 % in *tert*-butanol) was added. The reaction mixture was stirred for 30 min at room temperature, diluted with 30 ml dichloromethane and washed with 10 ml of a 10 % aqueous 25 sodium sulfite and then 10 ml water. The organic phase was dried with sodium sulfate and evaporated to dryness under reduced pressure. The residue was purified by column chromatography with silica gel with a stepwise gradient of 0 to 4 % methanol in dichloromethane. The products were isolated as colorless foams.

(i) 2',5'-dideoxyadenosine-3'-O-bis(S-acetyl-2-thioethyl)-phosphate

30 (Compound 1 in Fig. 4): Yield, 100 mg (0.19 mmol, 46 %); TLC: R_f = 0.37 (methylenechloride/methanol = 93:7, v/v), R_f = 0.16 (ethyl acetate/methanol = 95:5,

v/v). HPLC: Rt = 34.2 min. ^1H NMR (DMSO- d_6): δ = 8.35 and 8.16 (2s, 1H each, H-2 and H-8), 7.30 (br s, 2H, NH_2), 6.32 (t, J = 6.9 Hz, 1H, H-1'), 4.96-4.94 (m, 1H, H-3'), 4.23-4.19 (m, 1H, H-4'), 4.15-4.07 (m, 4H, CH_2O), 3.26-3.15 (m, 1H, H-2' partially obscured by t, J = 6.3 Hz, 4H, CH_2S), 2.64-2.57 (m, 1H, H-2''), 2.36 and 2.35 (2s, 3H each, 2 CH_3 acetyl), 1.34 (d, J = 6.4 Hz, 3H, CH_3). ^{31}P NMR (DMSO- d_6): δ = -1.47. Elemental analysis: $\text{C}_{18}\text{H}_{26}\text{N}_5\text{O}_7\text{PS}_2$ (519.54); calcd. C 41.61, H 5.04, N 13.48.

(ii) *2',5'-dideoxyadenosine-3'-O-bis(S-pivaloyl-2-thioethyl)-phosphate* (Compound 2 in Fig. 4): Yield, 190 mg (0.31 mmol, 75 %); TLC: R_f = 0.45 (methylenchloride/methanol = 93:7, v/v), R_f = 0.27 (ethyl acetate/methanol = 95:5, v/v). HPLC: Rt = 42.8 min. ^1H NMR (DMSO- d_6): δ = 8.56 and 8.35 (2s, 1H each, H-2 and H-8), 6.37 (t, J = 6.7 Hz, 1H, H-1'), 4.95 (m, 1H, H-3'), 4.25-4.22 (m, 1H, H-4'), 4.14-4.06 (m, 4H, CH_2O), 3.59 (br s, 2H, NH_2), 3.18-3.13 (m, 1H, H-2' partially obscured by t, J = 6.2 Hz, 4H, CH_2S), 2.68-2.64 (m, 1H, H-2''), 1.35 (d, J = 6.4 Hz, 3H, CH_3), 1.18 and 1.16 (2s, 9H each, 2 $\text{C}(\text{CH}_3)_3$). ^{31}P NMR (DMSO- d_6): δ = -1.47. Elemental analysis: $\text{C}_{24}\text{H}_{38}\text{N}_5\text{O}_7\text{PS}_2$ (603.70), calcd. C 47.75, H 6.34, N 11.60.

(iii) *2',5'-dideoxyadenosine-3'-O-bis(S-benzoyl-2-thioethyl)-phosphate* (Compound 3 in Fig. 4): Yield, 170 mg (0.26 mmol, 63 %); TLC: R_f = 0.33 (methylenchloride/methanol = 93:7, v/v), R_f = 0.19 (ethyl acetate/methanol = 95:5, v/v). HPLC: Rt = 41.6 min. ^1H NMR (DMSO- d_6): δ = 8.31 and 8.14 (2s, 1H each, H-2 and H-8), 7.92-7.87, 7.69-7.64, 7.56-7.48 (m, 10H, 2 C_6H_5), 7.30 (br s, 2H, NH_2), 6.30 (t, J = 6.9 Hz, 1H, H-1'), 4.97 (m, 1H, H-3'), 4.29-4.19 (m, 5H, H-4' and CH_2O), 3.41 (t, J = 6.1 Hz, 4H, CH_2S), 3.26-3.13 (m, 1H, H-2'), 2.62-2.54 (m, 1H, H-2''), 1.29 (d, J = 6.8 Hz, 3H, CH_3). ^{31}P NMR (DMSO- d_6): δ = -1.44. Elemental analysis: $\text{C}_{28}\text{H}_{30}\text{N}_5\text{O}_7\text{PS}_2$ (643.68); calcd. C 52.25, H 4.70, N 10.88.

25 **b. 2',5'-dideoxyadenosine-3'-O-cyclosaligenyl phosphates**

(compounds 10 and 11 in Fig. 4). To a stirred solution of 2',5'-dideoxyadenosine (141 mg, 0.60 mmol) in 10 ml dimethylformamide was added diisopropylethylamine (116 mg, 0.90 mmol, 1.5 eq) followed by the dropwise addition at -40°C of a solution of the cyclic chlorophosphane (0.90 mmol, 1.5 eq/unsubstituted: 170 mg (for compound 10); -3-methyl- 182 mg (for compound 11)) in 1 ml tetrahydrofuran as per Scheme 1

(Fig. 5). The reaction mixture was stirred for 30 min and *tert*-butylhydroperoxide (0.2 ml, mmol, eq, 90 % in *tert*-butanol) was added. The reaction mixture was stirred for 30 min at room temperature and evaporated to dryness under reduced pressure. The residue was purified by column chromatography with silica gel using a stepwise
 5 gradient of 0 to 4 % methanol in dichloromethane. The products were isolated as colorless foams.

(i) 2',5'-dideoxyadenosine-3'-O-cyclosaligenyl phosphate (Compound 10 in Figure 4). Yield, 190 mg (0.47 mmol, 79 %); TLC: $R_f = 0.29$ (methylenchloride/methanol = 93:7, v/v), $R_f = 0.16$ (ethyl acetate/methanol = 95:5, v/v). HPLC: $R_t = 33.2$ min. ^1H NMR ($\text{DMSO}-d_6$): $\delta = 8.69, 8.34, 8.32$ and 8.12 (s, 2H, H-2 and H-8, two diastereomers), $7.44-7.05$ (m, 5H, 3H C_6H_4 and NH_2), $6.89-6.73$ (m, 1H, 1H C_6H_4), $6.33-6.27$ (m, 1H, H-1'), $5.58-5.30$ (m, 2H, CH_2), 5.15 (m, 1H, H-3'), $4.26-4.15$ (m, 1H, H-4'), $3.23-3.17$ (m, 1H, H-2'), $2.68-2.61$ (m, 1H, H-2''), $1.34-1.24$ (m, 3H, CH_3). ^{31}P NMR ($\text{DMSO}-d_6$): $\delta = -9.56, -9.60$. Elemental analysis: $\text{C}_{17}\text{H}_{18}\text{N}_5\text{O}_5\text{P}$ (403.34); calcd. C 50.63, H 4.50, N 17.36.
 15

(ii) 2',5'-dideoxyadenosine-3'-O-cyclo-3-methyl-saligenyl phosphate (Compound 11 in Figure 4). Yield, 200 mg (0.48 mmol, 80 %); TLC: $R_f = 0.31$ (methylenchloride /methanol = 93:7, v/v), $R_f = 0.17$ (ethyl acetate/methanol = 95:5, v/v); HPLC: $R_t = 34.1$ min. ^1H NMR ($\text{DMSO}-d_6$): $\delta = 8.65, 8.33, 8.32$ and 8.12 (s, 2H, H-2 and H-8, two diastereomers), $7.30-7.03$ and 6.72 (m, 5H, 3H C_6H_3 and NH_2), $6.30-6.27$ (m, 1H, H-1'), $5.54-5.48$ (m, 2H, CH_2), $5.18-5.15$ (m, 1H, H-3'), $4.22-4.11$ (m, 1H, H-4'), $3.29-3.21$ (m, 1H, H-2'), $2.67-2.60$ (m, 1H, H-2''), $2.27-2.15$ (m, 3H, CH_3 saligenyl), $1.37-1.25$ (m, 3H, CH_3). ^{31}P NMR ($\text{DMSO}-d_6$): $\delta = -8.98, -9.02$. Elemental analysis: $\text{C}_{18}\text{H}_{20}\text{N}_5\text{O}_5\text{P}$ (417.36); calcd. C 51.80, H 4.83, N 16.78.
 20

25

c. β -L-2',3'-dideoxyadenosine-5'-O-bis(S-acyl-2-thioethyl)-phosphate (compounds 7-9 in Fig. 4). To a stirred solution of β -L-2',3'-dideoxyadenosine (44 mg, 0.187 mmol) in a mixture of tetrahydrofuran and dimethylformamide (1.5 ml, 2:1, v/v) was added 1*H*-tetrazole (39 mg, 0.56 mmol, 3.0
 30 eq) followed by the dropwise addition at room temperature of a solution of the

appropriate bis(S-2-hydroxyethylthioacyl) *N,N*-diisopropylphosphoramidite. For β -L-2',3'-dideoxyadenosine-5'-O-bis(S-pivaloyl-2-thioethyl)-phosphate (or β -L-2',3'-dd-5'-AMP-bis(tBu-SATE), compound 8 in Fig. 4) this was bis(S-2-hydroxyethylthiopivaloyl) *N,N*-diisopropylphosphoramidite (127 mg, 0.28 mmol, 1.5 eq) in 0.5 ml tetrahydrofuran. The reaction mixture was stirred for 45 min and then cooled to -40° C and *tert*-butylhydroperoxide (45 ml, 0.45 mmol, 2.4 eq, 90 % in *tert*-butanol) was added. The reaction mixture was stirred for 30 min at room temperature, diluted with 30 ml dichloromethane and washed with 10 ml of a 10 % aqueous sodium sulfite and then 10 ml water. The organic phase was dried with sodium sulfate and evaporated to dryness under reduced pressure. The residue was purified by column chromatography with silica gel using a stepwise gradient of 0 to 4 % methanol in dichloromethane. The product (compound 8) was isolated as a colorless foam. Syntheses of β -L-2',3'-dideoxyadenosine-5'-O-bis(S-acetyl-2-thioethyl)-phosphate (or β -L-2',3'-dd-5'-AMP-bis(Me-SATE), compound 7 in Fig. 4) and β -L-2',3'-dideoxyadenosine-5'-O-bis(S-benzoyl-2-thioethyl)-phosphate (or β -L-2',3'-dd-5'-AMP-bis(tBu-SATE), compound 9 in Fig. 4) follow from the respective bis(S-2-hydroxyethylthioacyl) *N,N*-diisopropylphosphoramidites in a similar fashion.

β -L-2',3'-dideoxyadenosine-3'-O-bis(S-pivaloyl-2-thioethyl)-phosphate (Compound 8 in Fig. 4). Yield, 60 mg (0.099 mmol, 53 %); TLC: R_f = 0.32 (methylene-chloride/methanol = 93:7, v/v), R_f = 0.14 (ethyl acetate/methanol = 95:5, v/v). HPLC: R_t = 41.1 min. ^1H NMR (DMSO- d_6): δ = 8.40 and 8.28 (2s, 1H each, H-2 and H-8), 7.96 (br s, 2H, NH_2), 6.28 (t, J = 5.2 Hz, 1H, H-1'), 4.31 (m, 1H, H-4'), 4.19-4.08 (m, 2H, 2H-3'), 4.01-3.93 (m, 4H, CH_2O), 3.06 (t, J = 6.1 Hz, 4H, CH_2S), 2.51 (m, 2H, 2 H-5'), 2.18-2.11 (m, 2H, 2 H-2'), 1.16 and 1.15 (2s, 9H each, 2 $\text{C}(\text{CH}_3)_3$). ^{31}P NMR (DMSO- d_6): δ = -0.59. Elemental analysis: $\text{C}_{24}\text{H}_{38}\text{N}_5\text{O}_7\text{PS}_2$ (603.70); calcd. C 47.75, H 6.34, N 11.60.

d. (R)-Bis(S-pivaloyl-2-thioethyl) 9-(2-phosphonylmethoxypropyl)adenine (Compounds 15-17 in Fig. 4). For the synthesis of the -pivaloyl- derivative (compound 16 in Fig. 4) a mixture of

monotriethylammonium *N*^ε-(4-monomethoxytrityl)-9-(2-phosphonylmethoxypropyl) adenine (86 mg, 0.13 mmol) and S-pivaloyl-2-thioethanol (105 mg, 0.65 mol, 5.0 eq) was coevaporated three times with 5 ml pyridine each and dissolved in 1.5 ml of dry pyridine. After addition of 1-(mesitylenesulfonyl)-3-nitro-1H-1,2,4-triazole (193 mg, 5 0.65 mmol, 5.0 eq) the reaction mixture was stirred for 3 d at ambient temperature. The reaction was stopped by addition of 20 ml saturated aqueous sodium bicarbonate and the resulting mixture was extracted with 30 ml methylenechloride. The organic phase was dried by filtration through MgSO₄ and evaporated to dryness. The residue was purified by flash chromatography (20 g silica gel) with a stepwise gradient of 0 to 10 4% methanol in methylenechloride. Appropriate fractions were combined and evaporated to dryness. The residue was dissolved in 5 ml of a mixture of acetic acid/water/methanol = 8:1:1 (v:v:v). The reaction mixture was stirred for 24 h at ambient temperature. The solvent was evaporated *in vacuo* and the residue was coevaporated twice with 5 ml toluene, twice with 5 ml methanol and then with 5 ml 15 methylenechloride. The residue was purified by flash chromatography (15 g silica gel) with a stepwise gradient of 0 to 20 % methanol in methylenechloride yielding 48 mg (R)-bis(S-pivaloyl-2-thioethyl) 9-(2-phosphonylmethoxypropyl)adenine (compound 16 in Fig. 4; 0.083 mmol, 64 %) as colorless oil. Synthesis of (R)-bis(S-acetyl-2-thioethyl)-9-(2-phosphonylmethoxy-propyl)adenine (compound 15 in Fig. 20 4) and (R)-bis(S-benzoyl-2-thioethyl)-9-(2-phosphonyl-methoxypropyl)adenine (compound 17 in Fig. 4) follow from the respective S-acyl-2-thioethanol in a similar fashion.

(R)-Bis(S-pivaloyl-2-thioethyl) 9-(2-phosphonylmethoxypropyl)adenine. (Compound 16 in Fig. 4) TLC: R_f = 0.28 (methylenechloride/methanol = 93:7, v/v), R_f = 0.08 (ethyl acetate/methanol = 95:5, 25 v/v). HPLC: Rt = 40.6 min. ¹H NMR (DMSO-*d*₆): δ = 8.14 and 8.06 (2s, 1H each, H-2 and H-8), 7.23 (br s, 2H, NH₂), 4.25-4.18 (m, 2H, NCH₂), 3.99-3.85 (m, 7H, CH₃CH, PCH₂, 2 CH₂O), 3.07-3.00 (m, 4H, CH₂S), 1.17 (s, 18H, 2 C(CH₃)₃), 1.09 (d, J = 6.2 Hz, 3H, CH₃). ³¹P NMR (DMSO-*d*₆): δ = 22.97; Elemental analysis: 30 C₂₃H₃₈N₅O₆PS₂ (575.69); calcd. C 47.99, H 6.65, N 12.17.

e. General methods for synthesis of compounds 1-3 and 7-17. ^{31}P

(101 MHz) and ^1H NMR (250 MHz): Bruker AC250; the residual solvent peaks were used as internal standard for the ^1H NMR spectra, phosphoric acid was used as external standard for the ^{31}P NMR spectra. All ^{31}P NMR spectra were recorded in the
5 proton-decoupled mode. Chemical shifts are given in δ (ppm) and coupling constants, J , are in Hz. Column chromatography was carried out on silica gel 60 (particle size 0.040-0.063 mm; EM Science, Merck, Darmstadt). Analytical thin-layer chromatography, used to monitor all reactions, was performed on silica gel 60 PF₂₅₄, with fluorescence indicator (0.2 mm; Merck, Darmstadt) on aluminum plates, and
10 visualization was accomplished by UV absorbance. Phosphorus containing compounds were detected by spraying with Hanes molybdate reagent. Sugar containing compounds were detected by spraying with anisaldehyde reagent each time followed by heating. High-performance liquid chromatography (HPLC) studies were carried out on a Waters system equipped with a model 600 controller, a model
15 717plus autosampler, a model 996 photodiode array detector and a Millennium data workstation. Separations were by reverse-phase chromatography (Ultrasphere, C18, 250x4.6 mm, 5 μm) with linear gradients of 0 to 100 % acetonitrile in water over a 40 min period at a flow rate of 1 ml/min. S-acyl-2-thioethanols and bis(S-2-hydroxyethylthioacyl) *N,N*-diisopropylphosphoramidites were synthesized according
20 to Lefebvre et al.[50]. The cyclic chlorophosphanes were synthesized according to Meier [51]. 2',5'-dideoxyadenosine was synthesized according to Désaubry et al. [52]. Monotriethylammonium *N*⁶-(4-monomethoxytrityl)-9-(2-phosphonylmethoxypropyl)adenine was synthesized according to Holy et al.[53,54]. Solvents: pyridine, tetrahydrofuran and dimethylformamide were analytical quality,
25 absolute from Fluka. 1*H*-tetrazole and 1-(mesitylenesulfonyl)-3-nitro-1*H*-1,2,4-triazole were from Aldrich.

f. Syntheses of 9-(2',5'-dideoxy- β -L-erythro-pentofuranosyl)adenine (β -L-2',5'-dideoxyadenosine) and its *tert*-Bu-SATE
30 (compound 5 in Fig. 4).

(i) **9-(2',5'-dideoxy- β -L-erythro-pentofuranosyl)adenine**. By use of the procedure previously described in the D-series by Désaubry et al. [52], β -L-2'-deoxyadenosine (0.7g, 2.8 mmol) was dissolved in pyridine (10 ml) and treated with diphenyldisulfide (0.76 g, 3.5 mmol) and tributylphosphine (0.87 ml, 3.5 mmol) (Scheme 2, Fig. 5). After 24 h, methanol (10 ml) was added, the solution was concentrated to dryness and coevaporated with toluene, then with methanol. Crude material was purified by silica gel column chromatography using a stepwise gradient of 0 to 12% methanol in dichloromethane to give pure thio intermediate (0.70 g, 73%). The thio intermediate (0.7 g, 2.04 mmol) was dissolved in a mixture of methanol-isopropanol (1/1, v/v, 10 ml) and treated with Raney Nickel (1 g). The suspension was refluxed for 10 days, then Nickel was removed and washed by three portions of boiling methanol. The combined filtrates were concentrated to dryness and the residue was purified by silica gel column chromatography with an isocratic gradient of 10% methanol in dichloromethane to give pure 9-(2,5-dideoxy- β -L-erythro-pentofuranosyl)adenine 1 (282 mg, 59%).

β -L-2',5'-dideoxyadenosine. R_f (MeOH/CH₂Cl₂, 15/85) = 0.33; mp : 173°C; UV (EtOH 95) λ_{\max} 260nm (ϵ , 14600), λ_{\min} 227nm (ϵ , 2200); ¹H NMR (DMSO-*d*₆) δ = 8.28 (s, 1H, H-8), 8.13 (s, 1H, H-2), 7.22 (br s, 2H, NH₂-6), 6.26 (t, 1H, H-1', *J* = 6.7 Hz), 5.30 (br s, 1H, OH-3'), 4.19 (m, 1H, H-3'), 3.9-3.8 (m, 1H, H-4'), 2.8-2.7 (m, 1H, H-2'), 2.3-2.2 (m, 1H, H-2''), 1.24 (d, 3H, CH₃-5', *J* = 6.4 Hz); ¹³C NMR (DMSO-*d*₆) δ = 156.9 (C-6), 153.4 (C-2), 150.0 (C-4), 140.3 (C-8), 120.0 (C-5), 83.6 (C-1'), 83.2 (C-4'), 75.5 (C-3'), 39.3 (CH₂-2'), 19.9 (CH₃-5'); MS FAB>0 *m/z* 236 (M+H)⁺, 136 (BH₂)⁺, MS FAB<0 *m/z* 234 (M-H)⁻; [α]_D²⁰ = - 39 (c, 0.96, DMSO); Anal. Calcd for C₁₀H₁₃N₅O₂ : C, 51.06; H, 5.57; N, 29.77.

(ii) **9-{2,5-dideoxy-3[bis(S-acyl-2-thioethyl)phosphate]- β -L-erythro-pentofuranosyl}adenine (β -L-2',5'-dd-3'-AMP-bis(acyl-SATE)** (compounds 4-6 in Figure 4). Adapting a published procedure for the synthesis of bis(*t*-butyl-SATE)pronucleosides [50], sublimed 1*H*-tetrazole (61 mg, 0.88 mmol) was added to a stirred solution of 9-(2,5-dideoxy- β -L-erythro-pentofuranosyl)adenine (β -L-2',5'-Ado; 69 mg, 0.29 mmol) in a mixture of tetrahydrofuran and

dimethylformamide (1.5 ml, 2/1, v/v), followed by the dropwise addition of a solution of bis(S-2-hydroxyethylthioacyl)-*N,N*-diisopropylphosphoramidite (-pivaloyl- 160 mg, 0.42 mmol, for compound 5 in Fig. 4) in tetrahydrofuran (0.5 ml) at room temperature. After 45 min, a 3 M solution of *tert*-butylhydroperoxide (0.293 ml, 0.88 mmol) in toluene was added. After further stirring at room temperature for 30 min, the mixture was diluted with dichloromethane and washed successively with 10% aqueous sodium sulfite solution and water. The organic layer was dried over sodium sulfate, filtered, and evaporated to dryness under reduced pressure. Purification of the residue by silica gel column chromatography using a stepwise gradient of 0 to 4% methanol in dichloromethane afforded 9-{2,5-dideoxy-3[bis(S-pivaloyl-2-thioethyl)phosphate]- β -L-erythro-pentofuranosyl}adenine (compound 5 in Fig 4.; 65 mg, 56%) as a colorless syrup. Syntheses of β -L-2',5'-dideoxyadenosine-3'-O-bis(S-acetyl-2-thioethyl)-phosphate (compound 4 in Fig. 4) and β -L-2',5'-dideoxyadenosine-3'-O-bis(S-benzoyl-2-thioethyl)-phosphate (compound 6 in Fig. 4) follow from the respective bis(S-2-hydroxyethylthioacyl)-*N,N*-diisopropylphosphoramidite in a similar fashion.

β -L-2',5'-dideoxyadenosine-3'-O-bis(S-pivaloyl-2-thioethyl)-phosphate (compound 5 in Fig. 4). R_f (MeOH/CH₂Cl₂, 10/90) = 0.39; UV (EtOH 95) λ_{max} 259 nm (ϵ , 14200), 236 nm (ϵ , 10300), λ_{min} 240 nm (ϵ , 10100); ¹H NMR (CDCl₃) δ = 8.28 (s, 1H, H-8), 7.87 (s, 1H, H-2), 6.30 (t, 1H, H-1', J = 6.8 Hz), 5.58 (br s, 2H, NH₂-6), 4.9-4.8 (m, 1H, H-3'), 4.3-4.2 (m, 1H, H-4'), 4.1-4.0 (m, 4H, 2'CH₂O), 3.2-3.0 (m, 5H, 2'CH₂S and H-2'), 2.7-2.6 (m, 1H, H-2''), 1.40 (d, 3H, CH₃-5', J = 6.6 Hz), 1.17 and 1.16 (2s, 18H, 2'(CH₃)₃C; ¹³C NMR (CDCl₃) δ = 206.1 (2'CO), 155.8 (C-6), 153.5 (C-2), 150.1 (C-4), 139.6 (C-8), 120.9 (C-5), 84.8 (C-1'), 82.1 (C-4'), 66.8 (2'CH₂O), 66.7 (C-3'), 47.0 (2'C(CH₃)₃), 38.1 (CH₂-2'), 28.9 (2'CH₂S), 27.7 (2'(CH₃)₃C), 19.6 (CH₃-5'); ³¹P NMR (CDCl₃) δ = - 2.05; MS FAB>0 m/z 604 (M+H)⁺, 136 (BH₂)⁺, MS FAB<0 m/z 458 (M-tBuSATE)⁻, 134 (B)⁻. HPLC: elution with a linear gradient of 0% to 80% acetonitrile in 20 mM triethylammonium acetate buffer (pH 7) programmed over 30 min at 1 ml/min, R_t = 25.8 min (99.67% area); isocratic elution with 45% acetonitrile in 20 mM triethylammonium acetate (pH 7) at

1 ml/min: Rt = 13.7 min (99.70% area). HRSM Calcd for $C_{24}H_{39}N_5O_7PS_2$ (M+H)⁺: 604.2113. Found: 604.2029.

iii) **General Methods for compounds 4-6.** 1H and ^{13}C NMR spectra were recorded at 400 MHz with proton decoupling, at ambient temperature, with a
5 Bruker DRX400. Chemical shifts are given in δ -values referenced to the residual solvent peak: chloroform ($CDCl_3$) at 7.26 ppm and 77.6 ppm or dimethylsulfoxide ($DMSO-d_6$) at 2.49 ppm and 39.5 ppm, relative to tetramethylsilane (TMS). Coupling constants, J , are reported in Hz. Deuterium exchange, decoupling and COSY experiments were performed in order to confirm proton assignments. 2D 1H - ^{13}C
10 heteronuclear COSY were recorded for the attribution of ^{13}C signals. ^{31}P NMR spectra were recorded at ambient temperature at 400 MHz with proton decoupling. Chemical shifts are reported relative to external H_3PO_4 . FAB mass spectra were recorded in the positive-ion and negative-ion modes on a JEOL SX 102 using thioglycerol/glycerol (1:1, v/v, G-T) as matrix. Melting points were determined in
15 open capillary tubes on a Gallenkamp MFB-595-010M apparatus and are uncorrected. UV spectra were recorded on a Uvikon 931 (Kontron) spectropolarimeter. Specific rotations were measured in a 1 cm cell on a Perkin-Elmer Model 241 spectropolarimeter settled on the sodium D line. Elemental analyses were carried out by the "Service de Microanalyses du CNRS, Division de Vernaison (France)." Thin
20 layer chromatography was performed on precoated aluminum sheets of silica gel 60 F_{254} (Merck, Art. 9885), visualization of products was accomplished by UV absorbance followed by charring with 10% ethanolic sulfuric acid with heating and/or by spraying with Hanes molybdate reagent. Column chromatography was carried out on silica gel 60 (Merck, Art. 9385). HPLC analysis was carried out using a reverse-
25 phase analytical column (Nucleosyl, C18, 150 x 4.6 mm, 5 μ m) equipped with a prefilter, a precolumn (Nucleosil, C18, 5 μ m) and a photodiode array detector (detection at 260 nm). All moisture-sensitive reactions were carried out under rigorous anhydrous conditions under argon atmosphere using oven-dried glassware. Solvents were dried and distilled prior to use and solids were dried over P_2O_5 under
30 reduced pressure at room temperature.

EXAMPLE 2**Effects of nucleotide prodrugs on [³H]cAMP formation in isolated cells.**

The nucleotide prodrugs were tested for their capacity to inhibit the forskolin-stimulated formation of [³H]cAMP in cells that had been preloaded with
5 [³H]adenine [47]. Human macrophages and two preadipocyte cell lines were tested.

Cell culture

(i) *Preadipocytes*: Preadipocytes were cultured in DMEM LG medium that was supplemented with 8 % fetal bovine serum and 1 % of an antibiotics mixture in 2 ml Petri dishes until they reached 90-95 % confluence. Growth medium was
10 removed and 1 ml of DMEM LG medium containing 2,8-[³H]-adenine (5 μ Ci) was added to each dish. Cells were incubated for 2 h at 37° C. Medium was removed and cells were incubated for 15 min at 37° C in 1 ml of unsupplemented medium. The medium was removed and 1 ml of DMEM LG medium supplemented with 0.1 mg/ml bovine serum albumin and 0.1 mM 3-isobutyl-1-methylxanthine was added.

15 (ii) *Macrophages*. Macrophages were plated on culture dishes and allowed to adhere in RPMI culture medium. Cells were labeled with 2,8-[³H]-adenine as described above for the preadipocytes.

Experimental treatment

Ten microliters of a solution of the test substance was added to yield
20 the appropriate concentration of inhibitor in the medium. After 15 min of incubation at 37° C 3 μ l of a 10 mM solution of forskolin in ethanol was added to the medium and the cells were further incubated for 15 min at 37°C. The medium was removed and the reaction was stopped by the addition of 1 ml of a freshly prepared solution of 0.5 mM solution of cAMP in 0.3 M perchloric acid. Dishes were then kept on ice for
25 30 min. To 800 μ l of the resulting cell extract was then added 90 μ l of 0.5 M triethanolammonium chloride buffer (pH = 7.5). The extract was then neutralized by addition of aqueous potassium hydroxide. [³H]cAMP was purified from a 700 μ l portion of this mixture by sequential chromatography on Dowex 50 and alumina as previously described [3]. A portion of the purified sample was used to quantify the
30 [³H]cAMP formed and the remainder was used to quantify recovery by UV

spectroscopy.

The expectation was that the nucleotide prodrug forms of these agents would enter cells, be deprotected, and be phosphorylated to the corresponding forms with three phosphates, which would then cause a potent inhibition of adenylyl cyclase (cf. Figure 3). It was additionally expected that the order of potency in intact cells would follow the potency of the phosphorylated ligands to inhibit adenylyl cyclase, that is PMEApp (IC_{50} 170 nM) < 2',5'-dd-3'-ATP (IC_{50} 40 nM) < β -L-2',3'-dd-5'-ATP (IC_{50} 24 nM) (Table 1). However, this was not the case and substantial selectivity of these nucleotide prodrugs was observed (Table 2 and Figure 6). Moreover, with both preadipocyte cell lines 2',5'-dd-3'-AMP-bis(t-Bu-SATE) (compound 2 in Fig. 4) caused accelerated rates of differentiation (not shown), similar to effects noted earlier with the precursor nucleoside, 2',5'-dd-Ado [40]. Furthermore, 2',5'-dd-3'-AMP-bis(t-Bu-SATE) (2) had no effect on the activity of adenylyl cyclases *per se*, whether enzyme was from rat brain or from the preadipocytes, whereas the cyclosalicyl-derivatives were weak inhibitors (Table 3). This latter weak inhibition was likely due to the spontaneous deprotection that the cyclosalicyl-derivatives undergo in aqueous solutions [51].

Table 2. Effects of various pro-nucleotides to inhibit forskolin-stimulated formation of [3 H]cAMP from cells prelabeled with [3 H]adenine.

Pro-Nucleotide		Ob1771 pre-adipocytes	F442A pre-adipocytes	Human Macrophages
		IC ₅₀ (nM)	IC ₅₀ (nM)	IC ₅₀ (nM)
5	β -D- 2',5'-dd-3'-AMP-bis(Me-SATE) (1)		30	
	β -D- 2',5'-dd-3'-AMP-bis(t-Bu-SATE)(2)	30	100	50
	β -D- 2',5'-dd-3'-AMP-bis(Ph-SATE) (3)		800	
	β -L-2',5'-dd-3'-AMP-bis(t-Bu-SATE) (5)		~2000	
	β -L-2',3'-dd-5'-AMP-bis(t-Bu-SATE) (8)	>30000		
10	t-Bu-SATE-PMEA (13)	>30000		
	t-Bu-SATE-PMPA (16)		>30000	
	β -D- 2',5'-dd-3'-AMP-HSal (10)	~2000		~2000
	β -D- 2',5'-dd-3'-AMP-MeSal (11)	~2000		
	(1R,2S)-9-(cyclopentyl)-Ade-2'-P-bis(t-	>30000		
15	Bu-SATE)(ns)			
	(1S,2R)-9-(cyclopentyl)-Ade-2'-P-bis(t-	>30000		
	Bu-SATE)(ns)			

Bold numbers in parentheses refer to structures shown in Figure 4; (ns) indicates the compound not shown. For comparison with the values given in Table 1, IC₅₀ values for inhibition of rat brain adenylyl cyclase by 2'-triphosphate forms of (1'R,2'S)- and (1'S,2'R)- enantiomers of 9-(cyclopentyl)-adenine were 450 nM and 9860 nM, respectively.

Table 3: Protected 2',5'-dd-3'-AMP does not inhibit isolated adenylyl cyclases (IC₅₀).

		Rat Brain Adenylyl Cyclase	Ob1771 cell membranes
	β -D- 2',5'-dd-3'-AMP-(H-Sal) (10)	7 μ M	30 μ M
	β -D- 2',5'-dd-3'-AMP-(Me-Sal) (11)	7 μ M	60 μ M
30	β -D- 2',5'-dd-3'-AMP-bis(t-Bu-SATE)(2)	no inhibition at 100 μ M	20 % inhibition at 100 μ M

Adenylyl cyclase activities were determined as previously described [24-32], with a reaction mixture containing 100 μ M 5'ATP, 5 mM $MnCl_2$, and 100 μ M forskolin. IC_{50} values were determined graphically.

5

The data suggest that the effectiveness of the nucleotide prodrugs in cells was not a function solely of the potency of their presumed triphosphate derivatives to inhibit adenylyl cyclases, but that efficacy may be dictated also by the processing of the prodrugs and the mechanisms by which inhibition occurs. The most effective agents were the prodrug precursors of 2',5'-dd-3'-ATP, a non-competitive, post-transition-state inhibitor. The prodrug precursors of β -L-2',3'-dd-5'-ATP and PMEA were ineffective in intact cells, despite the fact that these nucleotides were potent inhibitors of isolated adenylyl cyclase. β -L-2',3'-dd-5'-ATP is a pre-transition-state competitive inhibitor, and PMEA causes mixed inhibition [31]. The data are fully consistent with there being a preferential structure of nucleotide prodrug which acts solely on intact cells to cause an intracellular inhibition of adenylyl cyclase and a consequential shutdown of cAMP/PKA-mediated pathways.

15

EXAMPLE 3

20

Effect of β -D- 2',5'-dd-3'-AMP-bis(t-Bu-SATE) cardiac myocytes

25

The efficacy of 2',5'-dd-3'-AMP-bis(t-Bu-SATE) in isolated preadipocytes and macrophages (Table 2 and Figure 6) suggested that it should work in other tissues as well. Cardiac ventricular myocytes exhibit an isoproterenol-stimulated, cAMP/PKA-dependent Cl^- channel [48]. Cl^- channel function was also blocked by 2',5'-dd-3'-AMP-bis(t-Bu-SATE) (IC_{50} ~82 nM) (not shown). Thus, in three cell types and with two different end-points, this prodrug showed a striking potency to block stimulated responses, whether by hormone or by an agent acting directly on adenylyl cyclase.

30

EXAMPLE 4

Effects of β -D- 2',5'-dd-3'-AMP-bis(t-Bu-SATE) (2) on cardiac

function.

The blockade by 2',5'-dd-3'-AMP-bis(t-Bu-SATE) (2) of isoproterenol-stimulated Cl^- channel activity in ventricular myocytes suggested that this pronucleotide should also affect other aspects of cardiac function. To test this, rat
5 atria were excised from a male albino rat and attached to a strain gauge to allow contractions to be measured. The spontaneously contracting tissue was suspended in a chamber and allowed to equilibrate for 15 min in Krebs-Henseleit medium (Sigma K3753) supplemented with 25 mM NaHCO_3 , 2.5 mM CaCl_2 , and 2 mM pyruvate, maintained at 37° and continuously gassed with 95%/5% CO_2 . The stimulation of
10 contractility caused by phenylephrine (Figure 7) or forskolin (not shown) were completely blocked by preincubation with 10 μM 2',5'-dd-3'-AMP-bis(t-Bu-SATE) for 15 min (Fig. 8).

From the above examples it is likely that 2',5'-dd-3'-AMP-bis(t-Bu-SATE) will act in many cells and tissues to block regulation that is mediated by the
15 cAMP/PKA pathway. Potencies and efficacies may differ from those noted here (Table 2 and Figures 6 and 8) and this may be attributable to differences in expression levels and sensitivities of the various adenylyl cyclase isozymes. The expectation is that 2',5'-dd-3'-AMP-bis(t-Bu-SATE), 2',5'-dd-3'-AMP-bis(Me-SATE), 2',5'-dd-3'-
20 AMP-bis(Ph-SATE), and other nucleotide prodrugs that are similarly based on adenine nucleoside 3'-phosphates, but with different protecting groups, will constitute a family of agents that can be used to inhibit adenylyl cyclases in intact cells and tissues. It is important to note that 2',5'-dd-3'-AMP-bis(t-Bu-SATE), even at
concentrations up to 300 μM , had no effect on adenylyl cyclase isolated from these
25 tissues, i.e., effects of this nucleotide prodrug were elicited only in the intact cell. The expected selectivity of this group will be due to both the general potency of adenine nucleoside 3'-polyphosphates to inhibit adenylyl cyclases and to the fact that this group of ligands inhibit adenylyl cyclases by a non-competitive, post-transition state mechanism.

30 The 9-substituted adenine derivatives of the invention may include other rationally designed structures including, for example, equivalent of 3'-phosphate

or 3'-thiophosphate derivatives that are protected by groups that allow the resulting 3'-phosphate- or 3'-thiophosphate-containing ligand to enter cells and tissues. Although the emphasis here has been with derivatives of -S-acyl-2-thioethyl (-SATE) as protecting groups, other protecting groups are also to be included. These include, for example, -H(salicyl) and -methyl(salicyl) groups (cf. Figure 4), amino acids coupled through hydroxyl- or thio- groups, N-coupled amino acids, amines, sugars, amino sugars, lipids, fatty acids, glycols, nucleotides, nucleosides, fluorophores [34, 52], steroids (e.g., cholesteryl groups [34, 52]), dithioethyl derivatives [55], and derivatives of these. Many of these compounds will become useful and valuable agents for pharmacological and biochemical investigations involving intact cells, tissues, and organisms.

REFERENCES

- 1) Taylor, S. (1989) *J.Biol. Chem.* **264**: 8443-8446.
- 2) Francis, S.H., and Corbin, J.D. (1999) *Crit. Rev. Clin. Lab. Sci.* **36(4)**: 275-328.
- 3) Zagotta, W.N., and Siegelbaum, S.A. (1996) *Ann. Rev. Neurosciences* **19**: 235-263.
- 4) Finn, J.T., Grunwald, M.E., and Yau, K.W. (1996) *Ann. Rev. Physiol.* **58**: 395-426.
- 5) Sutherland, E.W., Rall, T.W., and Menon, T. (1962) *J.Biol. Chem.* **237**: 1220-1227.
- 6) Smit, M.J., and Iyengar, R. (1998) *Adv. Second Messenger & Phosphoprotein Res.* **32**: 1-21.
- 7) Butcher, R.W., and Sutherland, E.W. (1962) *J.Biol. Chem.* **237**: 1244-1250.
- 8) Beavo, J.A. (1995) *Physiological Rev.* **75**: 725-748.
- 9) Rodbell, M., Birnbaumer, L., Pohl, S.L., and Krans, H.M.J. (1971) *J. Biol. Chem.* **246**: 1877-1882.
- 10) Rodbell, M. (1995) *Angew. Chem. Int. Ed. Engl.* **34**: 1420-1428.
- 11) Gilman, A.G. (1995) *Angew. Chem. Int. Ed. Engl.* **34**: 1406-1419.

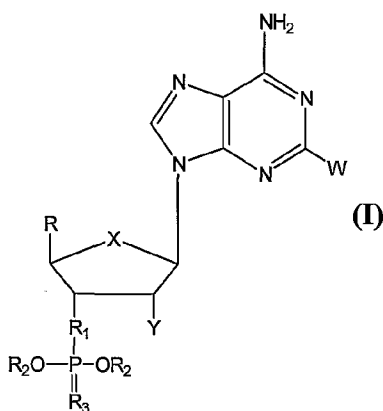
- 12) Brostrom, C.O., Huang, Y., Breckenridge, B.M., and Woolf, D.J. (1975) *Proc. Natl. Acad. Sci. USA* **72**: 64-68.
- 13) Seamon, K. and Daly, J.W. (1981) *J. Biol. Chem.* **256**: 9799-9801.
- 14) De Souza, N.J. (1993) *J. Ethnopharmacol.* **38**: 177.
- 5 15) Drummond, G.I. (1981) *Arch. Biochem. Biophys.* **211**: 30-38.
- 16) Moullet, O., and Dreyer, J.-L. N(1994) *Biochem. J.* **300**: 99-106.
- 17) Yoshimasa, T., Sibley, D.R., Bouvier, M., Lefkowitz, R.J., Caron, M.G. (1987) *Nature* **327**: 67-70.
- 18) Weinryb, I., and Michel, I.M. (1974) *Biochim. Biophys. Acta* **334**: 218-225.
- 10 19) Londos, C., and Wolff, J. (1977) *Proc. Natl. Acad. Sci. USA* **74**: 5482-5486.
- 20) Haslam, R.J., Davidson, M.M.L., and Desjardins, J.V. (1978) *Biochem. J.* **176**: 83-95.
- 21) Johnson, R.A., Saur, W. and Jakobs, K.H. (1979) *J. Biol. Chem.* **254**: 1094-1101.
- 15 22) Londos, C., Cooper, D.M.F., and Wolff, J. (1980) *Proc. Natl. Acad. Sci. USA* **77**: 2551-2554.
- 23) Florio, V.A., and Ross, E.M. (1983) *Molec. Pharmacol.* **24**: 195-202.
- 24) Johnson, R.A., Yeung, S.M.H., Stübner, D., Bushfield, M., and Shoshani, I. (1989) *Mol. Pharmacol.* **35**: 681-688.
- 20 25) Bushfield, M., Shoshani, I., and Johnson, R.A. (1990) *Molec. Pharmacol.* **38**: 848-853.
- 26) Johnson, R.A., and Shoshani, I. (1990) *J. Biol. Chem.* **265**: 11595-11600.
- 27) Yeung, S.-M.H., and Johnson, R.A. (1990) *J. Biol. Chem.* **265**: 16745-16750.
- 28) Désaubry, L., Shoshani, I., and Johnson, R.A. (1996) *J. Biol. Chem.* **271**: 2380-2383.
- 25 29) Désaubry, L., Shoshani, I., and Johnson, R.A. (1996) *J. Biol. Chem.* **271**: 14028-14034.
- 30) Désaubry, L., and Johnson, R.A. (1998) *J. Biol. Chem.* **38**: 24972-24977.
- 31) Shoshani, I., Boudou, V., Pierra, C., Gosselin, G., and Johnson, R.A. (1999) *J. Biol. Chem.* **274**: 34735-34741.
- 30

- 32) Shoshani, I., Laux, W.H.G., Périgaud, C., Gosselin, G., and Johnson, R.A. (1999) *J. Biol. Chem.* **274**: 34742-34744.
- 33) Dessauer, C.W., and Gilman, A.G. (1997) *J. Biol. Chem.* **272**: 27787-27795.
- 34) Johnson, R.A., Désaubry, L., and Shoshani, I. (1998) "Method and compounds for the inhibition of adenylyl cyclase. U.S. Pat. No. 5,795,756.
- 5 35) Johnson, R.A., Désaubry, L., and Shoshani, I. (2000) U.S. Application Serial, No. 09/071,779.
- 36) Tesmer, J.J.G., Sunahara, R.K., Johnson, R.A., Gosselin, G., Gilman, A.G., and Sprang, S.R. (1999) *Science* **285**: 756-760.
- 10 37) Tesmer, J.J.G., Dessauer, C.W., Sunahara, R.K., Murray, L.D., Johnson, R.A., Gilman, A.G., and Sprang, S.R. (2000) *J. Biol. Chem.* **275**: (In review).
- 38) Fain, J.N., Pointer, R.H., and Ward, W.F. (1972) *J. Biol. Chem.* **247**: 6866-6872.
- 39) Claus, T.H., Anand-Srivastava, M.B., and Johnson, R.A. (1982) *Molec. & Cell. Endocrinol.* **26**: 269-279.
- 15 40) Ibrahimi, A., Abumrad, N., Maghareie, H., Golia, M., Shoshani, I., Désaubry, L., and Johnson, R.A. (1999) *Am J. Physiol.* **276** (*Cell Physiol.* **45**): C487-C496.
- 41) Gärtner, R., Greil, W., Demharter, R., and Horn, K. (1985) *Molec. Cell. Endocrin.* **42**: 145-155.
- 42) Grega, D.S., and Macdonald, R.L. (1987) *J. Neuroscience* **7**(3): 700-707.
- 20 43) Reid, I.R., Lowe, C., Cornish, J., Grayh, D.H., and Skinner, S.J.M. (1990) *Am. J. Physiol.* **258** (*Endocrinol. Metab.* 21): E708-E714.
- 44) Dillingham, M.A., and Anderson, R.J. (1985) *J. Membrane Biol.* **88**: 277-281.
- 45) Thomas, C.A., Weinberger, O.K., Ziegler, B.L., Grteenberg, S., Schieren, I., Silverstein, S.C., and Khoury, J.E. (1997) *Blood* **90**: 3760-3765.
- 25 46) Benzaria, S., Pélicano, H., Johnson, R., Maury, G., Imbach, J.-L., Aubertin, A.-M., Obert, G., and Gosselin, G. (1996) *J. Med. Chem.* **39**: 4958-4965.
- 47) Salomon Y. (1991) *Methods Enzymol.* **195**: 22-28.
- 48) Hiraoka, M., Kawano, S., Hirano, Y., and Furukawa, T. (1998) *Cardiovascular Res.* **40**: 23-33.

- 49) Hoffman, B.B., and Lefkowitz, R.J., In: *The Pharmacological Basis of Therapeutics*, (Hardman, Limbird, Molinoff, Ruddon, and Gilman, Eds.) 9th Edition, pp. 199-248, McGraw-Hill, New York, 1996.
- 50) Lefebvre, I., Perigaud, C., Pompon, A., Aubertin, A.-M., Girardet, J.-L., Kirn,
5 A., Gosselin, G., and Imbach, J.-L. (1995) *J. Med. Chem.*, **38**: 3941-3950.
- 51) Meier, C. (1996) *Angew. Chem. Int. Ed. Engl.* **35**: 70-72.
- 52) Désaubry, L., Shoshani, I., Johnson, R.A. (1995) *Nucleosides & Nucleotides* **14**:
1453-1460.
- 53) Holy, A., and Masojidkova, M. (1995) *Collect. Czech. Chem. Commun.* **60**:
10 1196-1212.
- 54) Holy, A., and Rosenberg, I. (1987) *Collect. Czech. Chem. Commun.* **52**: 2801-
2809.
- 55) Perigaud, C., Gosselin, G., Lefebvre, I., Girardet, J.-L., Benzaria, S., Barber, I.,
Imbach, J.-L. (1993) *Bioorg. Med. Chem. Lett.* **3**:2521-2526.
- 15

WHAT IS CLAIMED IS:

- 1 1. A compound represented by formula (I)
- 2 wherein W is selected from the group consisting of H, halogen, azido and amino



- 3 group;
 - 4 X is selected from the group consisting of O, S, N(H) and C;
 - 5 Y is selected from the group consisting of H, hydroxy, C₁₋₄ alkyl, C₁₋₄ alkoxy and
 - 6 halogen;
 - 7 R is selected from the group consisting of H, hydroxymethyl, C₁₋₄ alkyl, C₁₋₄ alkoxy
 - 8 and halogen;
 - 9 R₁ is selected from the group consisting of O, NH and CH₂;
 - 10 R₂ represents a radical selected from the group consisting of -(CH₂)_n-S-C(O)-R₄ and
 - 11 -(CH₂)_n-S-S-R₄, where n=1-4 and R₄ is a C₁₋₄ alkyl or aryl group and R₄ is optionally
 - 12 substituted with a halogen, amino, N-alkylamino, N, N di-alkylamino or C₁₋₄ alkoxy
 - 13 group and wherein each of the R₂ radicals may be the same or different;
 - 14 R₃ is O or S; and
 - 15 when X is C the cyclopentyl ring so formed may be saturated or unsaturated.
- 1 2. The compound of claim 1 where W is a hydrogen atom, and R₂
 - 2 represents a radical selected from the group consisting of -CH₂-CH₂-S-C(O)-R₄ and
 - 3 -CH₂-CH₂-S-S-R₄, where R₄ is a C₁₋₄ alkyl or aryl group.

1 3. The compound of claim 2 where R_4 is a radical selected from the group
2 consisting of methyl, tert-butyl and phenyl.

1 4. The compound of any one of claims 1-3 where R is selected from the
2 group consisting of H, hydroxymethyl, methyl and methoxy.

1 5. The compound of any one of claims 1-3 where Y is selected from the
2 group consisting of H, hydroxy, methyl and methoxy.

1 6. The compound of any one of claims 1-3 comprising a sugar moiety
2 where X is O, Y is hydroxy, R is hydroxymethyl and R_1 is O.

1 7. The compound of claim 6 wherein the sugar moiety is selected from the
2 group consisting of ribose, xylose, lyxose and arabinose, in the D or L configuration..

1 8. The compound of any one of claims 1-3 where X is S.

1 9. The compound of any one of claims 1-3 where X is N(H).

1 10. The compound of any one of claims 1-3 where X is CH_2 .

1 11. The compound of claim 10 wherein the cyclopentyl ring is
2 unsaturated.

1 12. A compound selected from the group consisting of:
2 2',5'-dideoxy- β -(L)-adenosine 3'-monophosphate-bis(Me-SATE);
3 2',5'-dideoxy- β -(D)-adenosine 3'-monophosphate-bis(Me-SATE);
4 2'-deoxy- β -(L)-adenosine 3'-monophosphate-bis(Me-SATE);
5 2'-deoxy- β -(D)-adenosine 3'-monophosphate-bis(Me-SATE);
6 2',5'-dideoxy- β -(L)-adenosine 3'-monophosphate-bis(t-Bu-SATE);

- 7 2',5'-dideoxy- β -(D)-adenosine 3'-monophosphate-bis(t-Bu-SATE);
- 8 2'-deoxy- β -(L)-adenosine 3'-monophosphate-bis(t-Bu-SATE);
- 9 2'-deoxy- β -(D)-adenosine 3'-monophosphate-bis(t-Bu-SATE);
- 10 2',5'-dideoxy- β -(L)-adenosine 3'-monophosphate-bis(Ph-SATE);
- 11 2',5'-dideoxy- β -(D)-adenosine 3'-monophosphate-bis(Ph-SATE);
- 12 2'-deoxy- β -(L)-adenosine 3'-monophosphate-bis(Ph-SATE);
- 13 2'-deoxy- β -(D)-adenosine 3'-monophosphate-bis(Ph-SATE);
- 14 2',5'-dideoxy- β -(L)-adenosine 3'-methylene phosphate-bis(Me-SATE);
- 15 2',5'-dideoxy- β -(D)-adenosine 3'-methylene phosphate-bis(Me-SATE);
- 16 2'-deoxy- β -(L)-adenosine 3'-methylene phosphate-bis(Me-SATE);
- 17 2'-deoxy- β -(D)-adenosine 3'-methylene phosphate-bis(Me-SATE);
- 18 2',5'-dideoxy- β -(L)-adenosine 3'-methylene phosphate-bis(t-Bu-SATE);
- 19 2',5'-dideoxy- β -(D)-adenosine 3'-methylene phosphate-bis(t-Bu-SATE);
- 20 2'-deoxy- β -(L)-adenosine 3'-methylene phosphate-bis(t-Bu-SATE);
- 21 2'-deoxy- β -(D)-adenosine 3'-methylene phosphate-bis(t-Bu-SATE);
- 22 2',5'-dideoxy- β -(L)-adenosine 3'-methylene phosphate-bis(Ph-SATE);
- 23 2',5'-dideoxy- β -(D)-adenosine 3'-methylene phosphate-bis(Ph-SATE);
- 24 2'-deoxy- β -(L)-adenosine 3'-methylene phosphate-bis(Ph-SATE);
- 25 2'-deoxy- β -(D)-adenosine 3'-methylene phosphate-bis(Ph-SATE);
- 26 2',5'-dideoxy- β -(L)-adenosine 3'-iminophosphate-bis(Me-SATE);
- 27 2',5'-dideoxy- β -(D)-adenosine 3'-iminophosphate-bis(Me-SATE);
- 28 2'-deoxy- β -(L)-adenosine 3'-iminophosphate-bis(Me-SATE);
- 29 2'-deoxy- β -(D)-adenosine 3'-iminophosphate-bis(Me-SATE);
- 30 2',5'-dideoxy- β -(L)-adenosine 3'-iminophosphate-bis(t-Bu-SATE);
- 31 2',5'-dideoxy- β -(D)-adenosine 3'-iminophosphate-bis(t-Bu-SATE);
- 32 2'-deoxy- β -(L)-adenosine 3'-iminophosphate-bis(t-Bu-SATE);
- 33 2'-deoxy- β -(D)-adenosine 3'-iminophosphate-bis(t-Bu-SATE);
- 34 2',5'-dideoxy- β -(L)-adenosine 3'-iminophosphate-bis(Ph-SATE);
- 35 2',5'-dideoxy- β -(D)-adenosine 3'-iminophosphate-bis(Ph-SATE);
- 36 2'-deoxy- β -(L)-adenosine 3'-iminophosphate-bis(Ph-SATE); and
- 37 2'-deoxy- β -(D)-adenosine 3'-iminophosphate-bis(Ph-SATE).

1 13. A compound selected from the group consisting of:

- 2 9-(tetrahydrofuryl)-(1'R,3'S)-adenine-3'-phosphate-bis(Me-SATE);
3 9-(tetrahydrofuryl)-(1'R,3'R)-adenine-3'-phosphate-bis(Me-SATE);
4 9-(tetrahydrofuryl)-(1'R,3'S)-adenine-3'-phosphate-bis(t-Bu-SATE);
5 9-(tetrahydrofuryl)-(1'R,3'R)-adenine-3'-phosphate-bis(t-Bu-SATE);
6 9-(tetrahydrofuryl)-(1'R,3'S)-adenine-3'-phosphate-bis(Ph-SATE);
7 9-(tetrahydrofuryl)-(1'R,3'R)-adenine-3'-phosphate-bis(Ph-SATE);
8 9-(tetrahydrofuryl)-(1'R,3'S)-adenine-3'-(CH₂)-phosphate-bis(Me-SATE);
9 9-(tetrahydrofuryl)-(1'R,3'R)-adenine-3'-(CH₂)-phosphate-bis(Me-SATE);
10 9-(tetrahydrofuryl)-(1'R,3'S)-adenine-3'-(CH₂)-phosphate-bis(t-Bu-SATE);
11 9-(tetrahydrofuryl)-(1'R,3'R)-adenine-3'-(CH₂)-phosphate-bis(t-Bu-SATE);
12 9-(tetrahydrofuryl)-(1'R,3'S)-adenine-3'-(CH₂)-phosphate-bis(Ph-SATE);
13 9-(tetrahydrofuryl)-(1'R,3'R)-adenine-3'-(CH₂)-phosphate-bis(Ph-SATE);
14 9-(tetrahydrofuryl)-(1'R,3'S)-adenine-3'-(NH)-phosphate-bis(Me-SATE);
15 9-(tetrahydrofuryl)-(1'R,3'R)-adenine-3'-(NH)-phosphate-bis(Me-SATE);
16 9-(tetrahydrofuryl)-(1'R,3'S)-adenine-3'-(NH)-phosphate-bis(t-Bu-SATE);
17 9-(tetrahydrofuryl)-(1'R,3'R)-adenine-3'-(NH)-phosphate-bis(t-Bu-SATE);
18 9-(tetrahydrofuryl)-(1'R,3'S)-adenine-3'-(NH)-phosphate-bis(Ph-SATE); and
19 9-(tetrahydrofuryl)-(1'R,3'R)-adenine-3'-(NH)-phosphate-bis(Ph-SATE).

1 14. A compound selected from the group consisting of:

- 2 9-(cyclopentyl)-(1'R,3'S)-adenine-3'-phosphate-bis(Me-SATE)
3 9-(cyclopentyl)-(1'R,3'R)-adenine-3'-phosphate-bis(Me-SATE)
4 9-(cyclopentyl)-(1'R,3'S)-adenine-3'-phosphate-bis(t-Bu-SATE)
5 9-(cyclopentyl)-(1'R,3'R)-adenine-3'-phosphate-bis(t-Bu-SATE)
6 9-(cyclopentyl)-(1'R,3'S)-adenine-3'-phosphate-bis(Ph-SATE)
7 9-(cyclopentyl)-(1'R,3'R)-adenine-3'-phosphate-bis(Ph-SATE)
8 9-(cyclopentyl)-(1'R,3'S)-adenine-3'-(CH₂)-phosphate-bis(Me-SATE)
9 9-(cyclopentyl)-(1'R,3'R)-adenine-3'-(CH₂)-phosphate-bis(Me-SATE)
10 9-(cyclopentyl)-(1'R,3'S)-adenine-3'-(CH₂)-phosphate-bis(t-Bu-SATE)

- 11 9-(cyclopentyl)-(1'R,3'R)-adenine-3'-(CH₂)-phosphate-bis(t-Bu-SATE)
12 9-(cyclopentyl)-(1'R,3'S)-adenine-3'-(CH₂)-phosphate-bis(Ph-SATE)
13 9-(cyclopentyl)-(1'R,3'R)-adenine-3'-(CH₂)-phosphate-bis(Ph-SATE)
14 9-(cyclopentyl)-(1'R,3'S)-adenine-3'-(NH)-phosphate-bis(Me-SATE)
15 9-(cyclopentyl)-(1'R,3'R)-adenine-3'-(NH)-phosphate-bis(Me-SATE)
16 9-(cyclopentyl)-(1'R,3'S)-adenine-3'-(NH)-phosphate-bis(t-Bu-SATE)
17 9-(cyclopentyl)-(1'R,3'R)-adenine-3'-(NH)-phosphate-bis(t-Bu-SATE)
18 9-(cyclopentyl)-(1'R,3'S)-adenine-3'-(NH)-phosphate-bis(Ph-SATE) and
19 9-(cyclopentyl)-(1'R,3'R)-adenine-3'-(NH)-phosphate-bis(Ph-SATE).

20 15. A pharmaceutical composition for use in inhibiting adenylyl cyclase
21 when exposed to cells comprising a compound of claims 1-14.

1 16. A method of inhibiting an isolated preparation of adenylyl cyclase by
2 unprotected mono- or polyphosphorylated ligands, the method comprising the step of
3 contacting the ligands with the isolated adenylyl cyclase.

1 17. The use of a compound for reducing adenylyl cyclase activity in intact
2 cells and tissues comprising:
3 a) selecting a compound from any of claims 1-14; and
4 b) contacting the compound with cells or tissues that contain adenylyl cyclase
5 for a period of time and under such conditions such that the adenylyl cyclase
6 activity is reduced.

1 18. The use of claim 17 further comprising assessing the reduction of
2 adenylyl cyclase activity by assay for 3':5'-cAMP.

1 19. The use of claim 16 wherein the assay for 3':5'-cAMP is a biochemical
2 means of measuring tissue levels of 3':5'-cAMP selected from the group consisting of
3 protein kinase binding assays, radioimmunoassays, ELISA and fluorometric assays.

1 20. The use of claim 18 wherein the assay for 3':5'-cAMP is selected from
2 the group consisting of measuring the formation of [³H]3':5'-cAMP in cells prelabeled
3 with [³H]adenine and using intact cells in combination with fluorescent indicator
4 techniques that respond to changes in cell 3':5'-cAMP levels.

1 21. The use of a compound in regulating cardiac function or performance
2 by administering an effective amount of a compound from any one of claims 1-14,
3 singly, in combination, or in conjunction with other compounds.

1 22. The use of claim 21 wherein the regulated function or performance is
2 selected from the group consisting of heart rate, force of contraction, cardiac output
3 and work.

1 23. The use of a compound in regulating cardiovascular function by
2 administering an effective amount of a compound from any one of claims 1-14,
3 singly, in combination, or in conjunction with other compounds.

1 24. The use of claim 23 wherein the regulated cardiovascular function is
2 selected from the group consisting of alterations in vasodilation and vasoconstriction.

1 25. The use of a compound in altering the growth, development, or
2 differentiation of cells or tissues by administering an effective amount of a compound
3 of any one of claims 1-14, singly, in combination, or in conjunction with other
4 compounds.

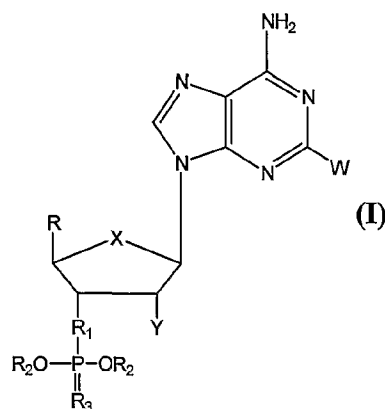
1 26. The use of claim 25 wherein administering the compound exhibits has
2 an angiogenic or antiangiogenic effect on blood vessels.

1 27. The use of claim 25 wherein administering the compound alters the
2 growth or character of benign or metastatic neoplasias or tumors.

1 28. The use of claim 25 wherein administering the compound alters *ex*
2 *vivo* regulation of cell or tissue growth, development or differentiation.

1 29. A compound for the use of inhibiting adenylyl cyclase when
2 administered to cells represented by formula (I)

3 wherein W is selected from the group consisting of H, halogen, azido and amino
4 group;
5 X is selected from the group consisting of O, S, N(H) and C;



6 Y is selected from the group consisting of H, hydroxy, C₁₋₄ alkyl, C₁₋₄ alkoxy and
7 halogen;
8 R is selected from the group consisting of H, hydroxymethyl, C₁₋₄ alkyl, C₁₋₄ alkoxy
9 and halogen;
10 R₁ is selected from the group consisting of O, NH and CH₂;
11 R₂ is selected from the group consisting of, -H(salicyl), -methyl(salicyl), amino acid,
12 amine, sugar, amino sugar, lipid, fatty acid, glycol, nucleotide, nucleoside, C₁₋₁₂ alkyl
13 and wherein each of the R₂ radicals may be the same or different;
14 R₃ is O or S; and
15 when X is C the cyclopentyl ring so formed may be saturated or unsaturated.

- 1 30. The compound of claim 29 where the amino acid is coupled to the
2 oxygen atom via a hydroxyl, thio, or amino group.

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 01/21523

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07H19/20 A61K31/7076 A61P9/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07H A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 795 756 A (JOHNSON ROGER A ET AL) 18 August 1998 (1998-08-18) the whole document ---	1, 15
A	R. VAN DER WOERD ET AL.: "Synthesis of P1,P2-dinucleotide pyrophosphates" TETRAHEDRON LETTERS, vol. 28, 1987, pages 2763-2766, XP002182704 the whole document ---	1
A	P.J.L.M. QUAEDFLIEG ET AL.: "Synthesis and conformational analysis of phosphate-methylated RNA dinucleotides" J. ORG. CHEM., vol. 56, 1991, pages 5846-5859, XP002182705 figure 1, scheme 3 --- -/--	1

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *Z* document member of the same patent family

Date of the actual completion of the international search

13 November 2001

Date of mailing of the international search report

03/12/2001

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

de Nooy, A

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/21523

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	IYER R P ET AL: "METHYL PHOSPHOTRIESTER OLIGONUCLEOTIDES: FACILE SYNTHESIS USING N-PENT-4-ENOYL NUCLEOSIDE PHOSPHORAMIDITES" JOURNAL OF ORGANIC CHEMISTRY, AMERICAN CHEMICAL SOCIETY. EASTON, US, vol. 60, no. 25, 15 December 1995 (1995-12-15), pages 8132-8133, XP002018376 ISSN: 0022-3263 scheme 2	1
A	D. LIPKIN ET AL.: "The base-catalysed alcoholysis of ribonucleic acids. A method for the determination of end groups" J. AM. CHEM. SOC., vol. 83, 1961, pages 4772-4780, XP002182706 the whole document	1

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 29,30

Present claims 29-30 relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT, however, is very feeble for the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search for those claims is impossible. Since no preferred embodiments could be identified in the application which are sufficiently clear, supported by the description and sufficiently disclosed to be susceptible of a meaningful search, the above mentioned claims have not been searched.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

In Original Application No
FBI, JS 01/21523

Form PCT/ISA/210 (patent family annex) (July 1992)